

## A multi-country comparison of caries-associated microflora in demographically diverse children

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**Objective** The aim of this formative international collaborative research on childhood dental caries was to undertake an initial investigation comparing the dental plaque of young children from diverse ethnic and socioeconomic backgrounds with and without dental caries. **Basic research design** The following four null hypotheses were investigated. There were no differences in numbers of individual taxa when comparing plaque samples from: 1) caries-free children from deprived and non-deprived backgrounds; 2) children from deprived and non-deprived backgrounds with at least 3 decayed teeth; 3) children from non-deprived backgrounds who are caries free with those from similar backgrounds with at least 3 decayed teeth; and, 4) children from deprived backgrounds who are caries free with those from similar backgrounds with at least 3 decayed teeth. **Participants** 277 children aged 3–4 years from 5 countries. **Main outcome measures** A sample of interproximal plaque from anterior teeth was collected using sterile dental floss, and cultured according to accepted international standards. **Results** Analysis of the data found that the first null hypothesis was accepted and that the fourth was rejected. Unexpectedly, the second null hypothesis was rejected as the children with caries from deprived and non-deprived backgrounds had a different caries-associated flora. In particular, children living in deprivation harbored more caries-associated bacteria [mutans streptococci and lactobacilli]. This greater microbial challenge was associated with a higher level of cavitated carious lesions and with more frequent consumption of confectionery. **Conclusions** Children from deprived backgrounds with caries may be further disadvantaged by having higher levels of caries-associated microflora.

*Key words:* caries-associated microflora, plaque, socio-economic status

### Introduction

The development of dental caries depends on oral bacteria metabolising dietary carbohydrates to produce acid. Although a wide spectrum of acidogenic flora may be involved, *Streptococcus mutans* has been most consistently identified as the most significant (Harris *et al.*, 2003). Lactobacilli and yeasts are commonly associated with caries and their presence has been associated with sugary diets (Beighton *et al.*, 1996). In young children acquisition and maintenance of a cariogenic microflora has been shown to relate initially to primary caregiver transmission and subsequently to sugary diets. Several studies have shown that some aspects of composition of the infant's flora reflect maternal flora (van Houte *et al.*, 1981). Caulfield *et al.* (1993) identified a window of infectivity from 19 to 31 months of age when children were most likely to become infected with *Streptococcus mutans* and develop early dental caries. Recent reports have found

colonisation in children less than 12 months old in high-risk populations (Karn *et al.*, 1998; Mohan *et al.*, 1998). While the flora is developing, its composition will change as teeth erupt (Berkowitz *et al.*, 1975; Könönen *et al.*, 1994; Mohan *et al.*, 1998). Enamel hypoplasia has been associated with high counts of mutans streptococci (Li *et al.*, 1994) and may predispose to caries when there are cariogenic bacteria with a high cariogenic diet (Seow, 1998). There are some studies investigating differences in dental caries and caries-associated microflora between ethnic groups (Zoitopoulos *et al.*, 1996), but information on pre-school populations is sparse. Less is known about whether children from disadvantaged and ethnically diverse families have a more virulent dental plaque. The aim of this study was to investigate whether the pattern of caries-associated taxa (mutans streptococci, lactobacilli, yeasts, and aciduric bacteria) is different in ethnically diverse children from deprived and non-deprived backgrounds with and without dental caries.

The hypotheses tested were that there were no differences in the numbers of individual taxa when comparing plaque samples from:

1. caries-free children from deprived and non-deprived backgrounds.
2. children from deprived and non-deprived backgrounds with at least 3 decayed teeth.
3. children from non-deprived backgrounds who are caries free with children from similar backgrounds with at least 3 decayed teeth.
4. children from deprived backgrounds who are caries free with children from similar backgrounds with at least 3 decayed teeth.

## Materials and Methods

### Subjects

Following ethical approval and informed consent, parents and children, aged 3 to 4 years, were recruited to a multi-centre international collaborative study of child dental health (Pine *et al.*, 2003). The main study comprised 2,822 children from 17 countries and investigators had been asked to recruit around 25 children in each of 4 groups: children with at least 3 decayed teeth from a) deprived and b) non-deprived families and caries free children from c) deprived and d) non-deprived families. From this larger study, investigators selected a sub-sample of children for collection of plaque samples. As this was a formative study and most differences were expected in children with caries, investigators were asked to collect plaque from 10 children in the caries groups and 5 children in the caries free groups. Children selected from the main groups were a sample of those seen in morning sessions to ensure maximum time for transportation of plaque.

### Dental plaque sampling

A standardised interproximal plaque sample was collected from an anterior site (maxillary anterior site) using sterile dental floss and placed into 1 ml of sterile PBSTC (1.58 g  $K_2HPO_4 \cdot 3H_2O$ , 0.34 g  $KH_2PO_4$  (anhydrous), 8 g NaCl, 0.00001 % CTAB stock, 1g sodium thioglycollate in 1 L water). The plaque samples were taken from a caries-free interproximal site between 52 and 53 or between 62 and 63 if this site was not present. If neither of these sites were available then 53/54 or 63/64 was sampled. The teeth were isolated to minimise salivary contamination. The samples were transported with cooling at 4°C within 48 hours to London (samples from USA, South Africa and UK) or Hong Kong (samples from Shanghai, Hong Kong and Singapore).

### Processing of plaque samples

Plaque samples were disaggregated using sterile beads and decimally diluted in PBSTC, 100 ml of appropriate dilutions were spread-plated (UK) or spiral-plated (Hong Kong) onto selective media for yeasts [Sabouraud Dextrose Agar (SAB), Oxoid, Basingstoke, Hants, UK.]; lactobacilli [Rogosa Agar (ROG), Oxoid]; mutans streptococci, *S. mutans* and *S. sobrinus* [Mitis Salivarius Agar supplemented with sucrose and bacitracin (BMSA); Becton Dickinson, Cowley, Oxon, UK] and Fastidious Anaerobe Agar (FAA, IPG Ltd, Bury, Lancs., UK.) supplemented with 5 % v/v horse blood for a total count.

The SAB was incubated aerobically for 3 days whereas the ROG and BMSA plates were incubated for 3 days anaerobically and the FAA plates for 7 days anaerobically. After incubation all plates were enumerated and a differential count of the BMSA plates was carried out to detect *S. mutans* and *S. sobrinus*-the identification of these organisms was confirmed using biochemical test as described previously (Beighton *et al.*, 1991).

Samples were also diluted to  $10^{-8}$  dilution to determine the number of aciduric organisms present in each using a most probable number (MPN) methodology as previously described (Alam *et al.*, 2000). A 15  $\mu$ l aliquot of each dilution (neat to  $-7$ ) was added to each of 12 wells of pH 4.8 trays; and each of  $-1$  to  $-8$  dilution to pH 5.2 and neutral trays containing 135  $\mu$ l of media as previously described. The MPN assays were incubated for 5 days anaerobically and the number of wells exhibiting growth at each dilution was recorded and the MPN of aciduric bacteria, at each pH were calculated.

### Analysis of data

Samples were assessed to determine the numbers, proportions or frequencies of isolation of individual taxa and all parameters are reported with statistical tests undertaken to determine the significance of any differences in numbers of micro-organisms. Similarly, the frequencies of isolation of lactobacilli, mutans streptococci and yeasts were also calculated for children according to country, site and ethnic group.

The raw numerical microbial counts were transformed to  $\log_{10}$  [colony forming unit + 1] per sample and counts of individual genera or species were expressed as a percentage of the total anaerobic count or for the MPN counts as a percentage of pH 7.0 count. Comparisons of counts between children were performed using Mann Whitney U-tests using the Bonferroni-Holm adjustment for the 4 hypotheses being tested, and the  $\chi^2$ -squared test was used to compare oral health, hygiene and dietary variables between groups of children.

## Results

### Profile of the children

This study included children from four racial groups in five countries providing a wide range of cultural environments (Table 1). Investigators at some sites found recruitment to this aspect of the study more complex leading to fewer numbers of children in some categories. However, in total, plaque was available for analysis from 267 children. Seventy-three children with caries from non-deprived families were included, 59 from similar backgrounds who were caries free. For children with caries from deprived families, 77 provided plaque samples with 58 from similar caries free children. The mean age of the children was 4.0 years and the mean dmft for those with caries was 3.8.

### Comparison of plaque composition for all children according to deprivation and caries status.

A summary of the data for all subjects according to deprivation and caries status is shown in Table 2. Deprived children with caries had the highest frequency of isolation for all the taxa, 75% of these children had

**Table 1.** Numbers of children by country, site, ethnic group, deprivation and caries status.

Country	Site	Ethnic group	Non-deprived with caries	Non deprived caries free	Deprived with caries	Deprived caries free
China	Hong Kong	Chinese	9	6	10	5
UK	Liverpool	Chinese	7	6	6	4
USA	San Francisco, CA	Chinese	5	2	13	9
China	Shanghai	Chinese	10	5	10	5
Singapore	Singapore	Chinese	9	7	12	10
UK	Dundee, Scotland	White	6	6	11	7
USA	San Antonio, TX	White	9	6		
USA	Washington, DC	African American	2	14	4	13
South Africa	Capetown	Cape Colored	16	7	11	5
Total			73	59	77	58

**Table 2.** Summary of the microbiological variables for all subjects according to deprivation and caries status; (cfu= colony forming units, MPN=most probable number).

	Non-deprived with caries		Non deprived caries free		Deprived with caries		Deprived caries free	
	Mean (S.E. mean)	Median	Mean (S.E. mean)	Median	Mean (S.E. Mean)	Median	Mean (S.E. Mean)	Median
<b>Log<sub>10</sub> (cfu+1) per sample</b>								
Total cfu	6.09 (0.11)	6.27	5.65 (0.18)	5.93	6.24 (0.13)	6.59	5.95 (0.15)	6.23
Mutans streptococci	2.09 (0.25)	1.71	1.48 (0.23)	0.00	3.00 (0.24)	3.53	1.42 (0.25)	0.00
Lactobacilli	0.49 (0.13)	0.00	0.20 (0.12)	0.00	1.66 (0.21)	1.18	0.16 (0.08)	0.00
Yeasts	1.03 (0.18)	0.00	0.91 (0.22)	0.00	1.36 (0.19)	0.00	0.61 (0.20)	0.00
MPN pH 7.0	4.75 (0.13)	4.52	4.61 (0.20)	4.32	5.10 (0.14)	5.25	4.91 (0.14)	5.08
MPN pH 5.2	3.09 (0.18)	3.20	2.66 (0.24)	2.71	3.56 (0.16)	3.84	3.39 (0.19)	3.63
MPN pH 4.8	1.69 (0.19)	1.32	1.49 (0.23)	1.08	2.10 (0.19)	2.20	1.68 (0.25)	0.94
<b>Individual taxa as a % of total cfu</b>								
<i>S. mutans</i>	0.02 (0.01)	0.00	<0.00 (0.00)	0.00	0.03 (0.01)	0.00	0.01 (0.00)	0.00
Lactobacilli	<0.00 (0.00)	0.00	0.01 (0.01)	0.00	0.02 (0.01)	0.00	<0.00 (0.00)	0.00
Yeasts	0.01 (0.00)	0.00	0.01 (0.00)	0.00	0.01 (0.01)	0.00	0.15 (0.14)	0.00
<b>% of MPN at pH 7.0 that also grow at acidic pHs</b>								
MPN pH 5.2	10.13 (2.61)	2.17	17.68 (13.18)	1.47	14.18 (3.20)	2.44	18.77 (6.30)	3.10
MPN pH 4.8	4.61 (1.91)	0.03	3.74 (1.71)	0.01	6.99 (2.53)	0.03	14.43 (7.88)	0.02
<b>Frequency of isolation (% of children with the taxa)</b>								
<i>S. mutans</i>	56		46		75		41	
Lactobacilli	21		5		54		7	
Yeasts	38		29		46		17	

mutans streptococci compared to only 41% of children from similar backgrounds who were caries free. The median value of mutans streptococci colony forming units was also the highest for deprived children with caries at 3.53, compared to 1.71 for non-deprived children with caries and zero for caries free children. Lactobacilli were isolated for 54% of children with caries from deprived backgrounds compared to only 7% of caries free children with the same family background. This low level of isolation was similar for non-deprived caries free children. In fact, overall the bacteriological profiles of the caries free children independent of background had many similarities.

In order to test the four null hypotheses, the significance of differences in the microbiological variables was tested and the results are presented in Table 3. No significant differences were found between caries free children, regardless of their deprivation status. Therefore, the first null hypothesis was not rejected. In contrast,

significantly more children from deprived backgrounds with caries had mutans streptococci ( $p=0.032$ ) and lactobacilli ( $p<0.001$ ) than non-deprived children with caries, leading to rejection of the second null hypothesis. The significant difference between non-deprived children with caries was that they were more likely to have lactobacilli ( $p=0.015$ ), but no other significant differences were found. The greatest differences were found between children living in deprivation, with more of those with caries having mutans streptococci ( $p<0.001$ ), lactobacilli ( $p<0.001$ ) and yeasts ( $p=0.005$ ) than those children who were caries free. This finding led to the rejection of the fourth null hypothesis.

#### *Plaque composition for children according to racial/ethnic group, deprivation caries status*

In general, fewer Chinese children living outside China had lactobacilli in their plaque. Similarly, fewer Chinese children in the UK were infected with mutans strepto-

**Table 3.** Significance of Mann-Whitney U-tests of differences in microbiological variables between children grouped by deprivation and caries status

	<i>Hypothesis 1: Caries free children: deprived vs non-deprived</i>	<i>Hypothesis 2: Children with caries: deprived vs non deprived</i>	<i>Hypothesis 3: Non deprived children: Caries free vs caries</i>	<i>Hypothesis 4: Deprived children: Caries free vs caries</i>
Total cfu	n.s.	n.s.	n.s.	n.s.
Mutans streptococci	n.s.	$p=0.032^1$	n.s.	$p<0.001^2$
Lactobacilli	n.s.	$p<0.001^1$	$p=0.015^2$	$p<0.001^2$
Yeasts	n.s.	n.s.	n.s.	$p=0.005^2$
MPN pH 7.0	n.s.	n.s.	n.s.	n.s.
MPN pH 5.2	n.s.	n.s.	n.s.	n.s.
MPN pH 4.8	n.s.	n.s.	n.s.	n.s.

n.s.=non significant at the 5% level

<sup>1</sup>deprived children > non-deprived children

<sup>2</sup>children with caries > caries free children

**Table 4.** Comparisons of frequencies (%) of isolation of *lactobacilli*, mutans streptococci and yeasts for children with and without caries from deprived and non-deprived socio-economic backgrounds by country and site.

Country	Site	Ethnic group	n	Non-deprived with caries	Non deprived caries free	Deprived with caries	Deprived caries free
<b>Lactobacilli</b>							
China	Hong Kong	Chinese	30	44	33	30	20
China	Shanghai	Chinese	30	40	0	70	20
Singapore	Singapore	Chinese	38	11	0	83	0
UK	Liverpool	Chinese	23	14	0	17	<u>25</u>
USA	San Francisco, CA	Chinese	29	20	<u>0</u>	33	0
UK	Dundee	White	30	17	0	36	0
USA	San Antonio, TX	White	15	0	0		
USA	Washington, DC	African American	33	<u>0</u>	0	<u>50</u>	8
South Africa	Capetown	Cape Coloured	39	19	14	91	0
<b>Mutans streptococci</b>							
China	Hong Kong	Chinese	30	56	50	90	40
China	Shanghai	Chinese	30	60	60	70	40
Singapore	Singapore	Chinese	38	56	57	58	50
UK	Liverpool	Chinese	23	14	0	33	<u>25</u>
USA	San Francisco, CA	Chinese	29	60	<u>100</u>	92	56
UK	Dundee	White	30	33	17	64	29
USA	San Antonio, TX	White	15	67	33		
USA	Washington, DC	African American	33	<u>50</u>	43	<u>75</u>	31
South Africa	Capetown	Cape Coloured	39	75	86	100	60
<b>Yeasts</b>							
China	Hong Kong	Chinese	30	22	17	50	20
China	Shanghai	Chinese	30	60	40	40	40
Singapore	Singapore	Chinese	38	11	14	25	30
UK	Liverpool	Chinese	23	29	17	17	<u>0</u>
USA	San Francisco, CA	Chinese	29	40	<u>100</u>	67	0
UK	Dundee	White	30	17	0	36	0
USA	San Antonio, TX	White	15	22	0		
USA	Washington, DC	African American	33	<u>100</u>	43	<u>50</u>	31
South Africa	Capetown	Cape Coloured	39	63	57	73	0

Underlined values represent cells with less than 5 cases

cocci irrespective of their deprivation or caries status. Overall, of the Chinese children more of those living in Shanghai had lactobacilli and yeasts. Levels of those with mutans streptococci were similar in Chinese children in Hong Kong, Shanghai and San Francisco. Twice as many white children with caries had mutans streptococci than those who were caries free. This finding was similar for non-deprived Chinese children living in the UK but not those in China or Singapore where infectivity levels were similar at around 50 to 60% irrespective of caries status. Cape colored children with caries living in depriva-

tion in Capetown had the highest numbers with lactobacilli, mutans streptococci and yeasts in their plaque.

#### *The relationship between oral hygiene, diet, caries and deprivation status*

All children who provided a plaque sample also had a dental examination to record caries status of each tooth and the presence of plaque on upper anterior teeth was recorded as an indicator of oral hygiene. Further, the parent/guardian of each child completed a detailed questionnaire. This aspect of the analysis sought to explore

**Table 5.** Comparisons of oral health, oral hygiene and dietary variables between caries and caries free, deprived and non-deprived groups, with significance tests between the non-deprived and deprived groups with caries.

	<i>Non-deprived with caries</i>	<i>Non deprived caries free</i>	<i>Deprived with caries</i>	<i>Deprived caries free</i>	<i>p-value (caries vs non caries)</i>	<i>p-value (non-deprived with caries vs deprived with caries)</i>
% with plaque on upper anterior teeth	81	65	81	77	n.s	n.s.
% with cavitated carious lesions	42	0	58	0	Not applicable	0.038
% eat sweets or chocolate every day or most days	62	50	79	41	<0.001	0.043
% eat other sugary foods every day or most days	66	51	67	33	<0.001	n.s.
% drink sugary drinks every day or most days	49	38	61	28	<0.001	n.s.
% add sugar to drinks	31	16	32	15	0.003	n.s.

n.s.=non significant.

whether some of the significant differences in plaque composition found when comparing children with caries from non-deprived and deprived families were occurring against a background of differences in oral hygiene and dietary habits namely sugary snack foods and drinks. Table 5 presents the data by group. As would be expected, more parents of children with caries reported that their child regularly consumed sweets and chocolates, other sugary foods, and drank sugary drinks ( $p<0.001$ ) than their caries free peers. For children with caries, significant differences were found for two parameters for children with caries from deprived and non-deprived families. Children with caries from deprived families had more cavitated carious lesions ( $p=0.038$ ) and were more likely to eat sweets and chocolates every day ( $p=0.043$ ).

### Discussion

In this study interproximal plaque samples were taken from pre-school children resident in nine different locations in order to obtain microbiological data to compare the broad relationship between the caries-associated microflora [aciduric bacteria, yeasts, lactobacilli and mutans streptococci], caries and socio-economic status in children from different ethnic groups. Interproximal plaque samples were collected from caries free and caries active (dmft>2) children from both deprived and non-deprived backgrounds. This study has highlighted that race/ethnicity and cultural setting may also impact on the composition of the flora. The populations investigated were diverse and although a common, post hoc classification of deprivation by maternal education was used, the impact of this type of “deprivation” will be relative within and across each population. Despite this caveat it is surprising how the associations between the caries-associated microflora and deprivation were consistent across the different groups of children.

The caries free children harbored a very similar plaque flora irrespective of their deprivation status, which is to be expected since caries initiation requires both plaque accumulation and ecological changes mediated by the

consumption of fermentable carbohydrates. In these children it was therefore postulated that plaque control was likely to be better and the intake of fermentable dietary carbohydrates lower and this was found to be the case. Conversely, it was unexpected that there should be differences in the flora of the caries active children related to deprivation. Two key underlying differences between these children were that the deprived children had more cavitated lesions and consumed more fermentable carbohydrate. These differences were apparent in the individual population groups studied. Investigators at most sites reported difficulty in finding children from non-deprived backgrounds with caries and for these children, the disease was less advanced, hence the significant difference in the prevalence of cavitated lesions and in part, the reduced bacterial counts.

There were few differences between the deprived caries active and caries free children but the major difference, which was apparent, was the higher numbers and greater proportion of lactobacilli in the plaque samples from the caries active children. These differences were not apparent in the individual populations, which might be a reflection of the small numbers of children in some groups. It has previously been reported that salivary and plaque levels of lactobacilli and mutans streptococci are significantly associated with caries in young children. Other data also indicate a higher prevalence of yeasts belonging to *Candida* species in children of ethnic Chinese origin. (Sedgley *et al.*, 1997). Due to the design, this study was able to separate out the effects of caries and deprivation on plaque composition. When deprivation was controlled for, the median values for mutans streptococci per sample were significantly different for children with caries from deprived families but not for non-deprived children with caries.

When the deprived caries free and caries active groups are considered there were a great many differences in the caries associated flora of the two populations. Given our understanding of the aetiology of dental caries, the full study data were able to confirm that these differences in plaque were reflecting a background of significant differences in the consumption of fermentable dietary

carbohydrates in association with poorer plaque control.

These data collectively indicate that, within the limitations of the small sample sizes, different consortia of caries-associated microorganism in interproximal plaque samples are not only related to the caries status of the individual but also to their deprivation status. In effect, children from deprived backgrounds with caries may be further disadvantaged by having higher levels of caries-associated microflora. In conclusion, this formative research has allowed an initial exploration of some fundamental hypotheses and indicated important directions for future research in this area clarifying the impact of deprivation on diverse populations of children who develop caries.

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